
A SURVEY OF MICROBIAL DIVERSITY¹

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ABSTRACT

The development of new technologies is increasing our understanding of prokaryotic and eukaryotic microbial diversity in terms of abundance, diversity, and ecological function. New studies reveal greater diversity than was previously known and have highlighted the importance of these organisms in ecosystem function. There is no consensus on the phylogenetic relationships of the various unicellular organisms at the phylum and kingdom level, and, as long as new taxa and characters are discovered, innovations in classifications of the kingdoms will likely continue. Some general conclusions are already clear. First, it is obvious that classifications that divide organisms into just two, three, four, or five kingdoms are too simplistic. In all of these simple schemes, one or more of the kingdoms ultimately contains a heterogeneous group of diverse taxa and is either paraphyletic or polyphyletic. Alternatively, a multi-kingdom system provides a more realistic view of the diversity of life. Concomitant with this is the realization that some traditional categories such as monera, algae, protozoa, or fungi can no longer be considered distinct phylogenetic groups. An overall scheme of classification that reflects our growing databases will hardly look like those followed in many textbooks, but it will reflect more accurately the relationships of the unicellular microorganisms.

Today, most people are aware of the economic and ecological importance of multicellular animals, plants, and fungi. But this appreciation does not usually extend to the importance of biological diversity of microorganisms. Possibly this is due to the scale at which most microorganisms live. The majority are too small to observe with the naked eye. Many are able to move and constantly change position and so cannot be easily tracked. They have short lives ranging from a few hours to a few days, but they can also form protective cysts and remain in the environment in an inactive state for years. Microorganisms can respond rapidly to changing conditions in their environment, becoming active and growing, or inactive and encysted within minutes or a few hours. All of this makes them more difficult for scientists to study and for many people to relate to and understand. Nevertheless, awareness of microbial diversity is important both because humans depend on microbes for many ecological services (production of oxygen, mineral and nutrient cycling, etc.) and because some forms are agents of disease. It is the purpose of this paper to try to convey something about the diversity of the microbial world: what is known and what is uncertain, and the prospects and challenges for surveying microbial diversity.

SCOPE OF MICROBIAL DIVERSITY

Microorganisms include both prokaryotes and unicellular eukaryotes (the protists). They are ubiquitous and in great abundance in all natural water,

sediment, or soil, and are in symbiotic association with multicellular organisms. Information about the size and nature of microbial populations is very incomplete because of difficulties in detecting and extracting them from the environment. Nevertheless, by piecing together various sources of information (and taking into account that activity and biomass measurements can be artifacts of the methods used) we are beginning to understand microbial diversity in terms of abundance, diversity, genetic variability, and ecological function.

ABUNDANCE

Microbial populations may show marked differences over very small distances (McAlice, 1970; Krumbein, 1971; Ashby & Rhodes-Roberts, 1976) or change in intervals as short as a few minutes during environmental flux (Erkenbrecher & Stevenson, 1975). Even taking this into account, in many environments numbers of microbial cells are high. For example, Fenchel (1992) recently estimated that a one-centimeter core of coastal marine sediment contained 4×10^{10} bacterial cells, 10^4 heterotrophic flagellates and amoebae, 10^8 chlorophyll *a*-containing microorganisms, and 2000 ciliates.

DIVERSITY

Microbial communities can also be made up of many different species. Many eukaryotic microorganisms can be identified by a taxonomic expert using standard microscopical methods (e.g., Fois-

¹ Support from U.S. National Science Foundation grant (DEB-9305925) is gratefully acknowledged.

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sner, 1991). Using silver stains and light microscopy, for example, Finlay et al. (1993) studied the kinds of ciliated protists (phylum Ciliophora) inhabiting the sandy sediment of a Spanish stream. They sampled this stream on just one day in winter (water temperature was 4°C at the warmest time of day). They marked out 1 square meter, and took from it 13 random sediment samples by pushing a 3-cm-diameter tube 3 cm into the sand, capping it, and extracting the sand. From this small amount of sand, they identified 65 species of ciliates belonging to 50 genera, from 17 orders. Considering the broad diversity of ciliate habitats available within the area, the importance of physical transport processes in the river basin, and the fact that many ciliate species have a cosmopolitan distribution, it is probable that the species richness they recorded is representative of the sandy sediment of the river in winter.

Analysis of the diversity of prokaryotic microorganisms is more difficult because they often lack enough morphological complexity to be accurately identified using microscopic methods. Traditionally, culturing techniques were used to recover prokaryotes from the environment and to characterize them by their physiological and nutritional requirements. Because none of these culturing methods permit the isolation and characterization of all prokaryote species, microbiologists attempted to obtain meaningful diversity data from the use of a wide range of techniques, each of which permits the study of a small fraction of the total marine biomass (Austin, 1988; Button et al., 1993). Even so, only a small percentage of the prokaryotes that actually exist in a habitat are revealed by these methods (Brock, 1987; Staley & Konopka, 1985).

More recently, modern molecular genetic techniques have enabled microbiologists to detect more microbial taxa (Stahl et al., 1989; Bull et al., 1992; Embley & Stackebrandt, 1994; Amann et al., 1995). These molecular tools allow microbiologists to extract and analyze nucleic acid sequences (primarily rRNA sequences) directly from the environment, thus circumventing the need to culture prokaryotes before identifying them. The first studies characterizing prokaryotes directly from environmental samples used sequences of electrophoretically separated 5S rRNA molecules (Stahl et al., 1984; Lane et al., 1985; Stahl et al., 1985). These techniques were refined for 16S rRNA (Olsen et al., 1986) and made easier to perform with the advent of polymerase chain reaction (PCR) technology. This modified approach was used by, for example, Giovannoni et al. (1990) to characterize the diversity of bacterioplankton in the Sargasso Sea and

revealed evidence for a previously unrecognized photosynthetic bacterium and a unique heterotrophic bacterial group.

An alternative molecular approach involves cloning cDNA transcribed from 16S rRNA using a primer complementary to the universally conserved region of rRNA (Ward et al., 1992; Weller & Ward, 1989). Ward et al. (1990) used this method to study the thermophilic (~50°C) microbial community from Octopus Spring in Yellowstone National Park. Although this community had been previously studied by more traditional methods and was believed to be relatively simple (Ward et al., 1987), the analysis of the cDNA revealed eight distinctive bacterial groups that did not match sequences known for cultured species including those previously isolated from hot springs.

Since these first studies, such molecular methods have facilitated our exploration of the free-living diversity of marine and estuarine waters (e.g., Schmidt et al., 1991; DeLong, 1992), organisms colonizing solid surfaces (Amann et al., 1992), organisms in water treatment activated sludge (Wagner et al., 1993), and organisms in soil (Torsvik et al., 1990; Masters et al., 1991; Stackebrandt et al., 1993). These methods have also been used to describe microorganisms living in association with other organisms, such as the chemoautotrophic bacteria symbiotic in invertebrates living near hydrothermal vents (Stahl et al., 1984; Distel et al., 1988), the cellulolytic nitrogen-fixing symbiotic bacteria that enable shipworms (wood-boring molluscs) to use wood as a principal source of food (Distel et al., 1991), the prokaryotic symbionts of various protists (Embley et al., 1992; Springer et al., 1992; Embley et al., 1993), the bioluminescent bacterial symbionts of fish (Haygood & Distel, 1993), and the cellulolytic symbionts in the rumen of mammalian herbivores (Angert et al., 1993), and to identify both plant and animal pathogens (e.g., Relman et al., 1990; Gundersen et al., 1994).

Caution must be used in interpreting results because the new molecular methods can be problematic. In addition to technical problems, such as probe sensitivity in certain conditions, it can be difficult to retrieve sequences of rare organisms (Amann et al., 1995). Furthermore, PCR probing of mixed DNA samples can suggest the presence of nonexistent organisms by forming chimeric sequences assembled from sequences of different species (Liesack et al., 1991). Nevertheless, it is obvious that molecular techniques provide extremely useful tools for analysis of microbial diversity (Stahl & Kane, 1992).

ECOLOGICAL FUNCTION

Microorganisms fill diverse ecological roles, and practically all key environmental processes are driven by microbial diversity. Microorganisms are essential to biological nutrient cycling, sulfur oxidation and reduction, ammonification, methanogenesis, and methane oxidation. Symbiosis with microorganisms is a major route by which many multicellular eukaryotes have gained access to complex metabolic activities (Douglas, 1994). These include nitrogen fixation, production of essential amino acids and vitamins, cellulose degradation, and photosynthesis. Microorganisms are essential to all major food webs. A breakdown of the annual carbon fixation and oxygen production by an individual photosynthetic microbe group is not available. It has been estimated that up to as much as 80% of the production of the open ocean is contributed by photosynthetic protists (Platt et al., 1983; Takahashi & Beinfang, 1983; Andersen, 1992), and in lakes chrysophytes (protists) and cyanobacteria contribute up to 40% of the primary productivity (Konopka, 1983).

In ecosystems that lack direct photosynthetic input, microorganisms are even more important as primary producers. Chemoautotrophic bacteria in environments such as deep sea vents and marine sediments provide energy to support a rich assemblage of heterotrophic microbes and animals (Jannasch & Taylor, 1984; Cavanaugh, 1994).

Despite the enormous production of autotrophic microbes, continual grazing by heterotrophs keeps their numbers in check. Some of the biomass produced by photo- and chemo-autotrophic microorganisms is consumed directly by animals (e.g., the echinoderm *Holothuria atra* obtains 10–40% of its carbon from bacteria; Moriarty et al., 1985), but the majority is consumed by heterotrophic microorganisms either from pools of dissolved organics, or as decomposers and primary consumers (Pomeroy, 1974; Williams, 1981; Fenchel, 1982, 1986, 1987; Azam et al., 1983; Ducklow, 1983; Hagström, 1984). These heterotrophs include prokaryotes, protists, and their protistan predators (Azam et al., 1983; Pomeroy, 1984; Williams, 1984; Sherr & Sherr, 1988). Ciliated protists (phylum Ciliophora) are especially important trophic links in these microbial food webs in that they are major consumers of planktonic bacteria, pico- and nano-planktonic autotrophs, diatoms, dinoflagellates, other ciliates, and heterotrophic flagellates and amoebae, and they are eaten in turn by animals such as zooplankton and planktivorous larval fish (reviewed in Pierce & Turner, 1992).

SYSTEMATIC DIVERSITY

As more is being learned about microbial diversity, it is becoming clear that commonly used classification schemes (e.g., Jahn & Jahn, 1949; Whittaker, 1969, 1977; Margulis & Schwartz, 1988) are oversimplified and simply inadequate for describing the true nature of the diversity, and that multiple kingdoms are needed (Leedale, 1974; Taylor, 1978; Lipscomb, 1985, 1989, 1991; Woese et al., 1990). Certainly the changes and rapid proliferation of new classification schemes of microorganisms are confusing to many, but they represent an invigorating period in microbial research from which a new, and hopefully more realistic and stable, system of microbial classification will emerge.

PROKARYOTE SYSTEMATIC DIVERSITY

In contrast to plants, animals, and even eukaryotic microorganisms, the morphology of prokaryotic microorganisms is, in general, too simple to serve as a basis for classification (Olsen & Woese, 1993; Amann et al., 1995). Classification traditionally depended on isolation by culturing followed by characterizing organisms according to physiological and biochemical traits. However, much of our understanding of prokaryote phylogeny is quite recent, due largely to the recent advent of modern molecular genetic sequencing methods. Sequences from ribosomal RNA, for example, have revealed two distinct groups of prokaryotes. This distinctiveness caused Woese et al. (1990) to propose a new superkingdom category, the domain, and to create three domains: Archaea (for the archebacteria), Bacteria, and Eucarya (for the eukaryotes). Despite criticism of this system (e.g., Cavalier-Smith, 1993), it is in widespread use today.

Archaea and Bacteria are both prokaryotic, but the archebacteria have no peptidoglycan in their cell wall, have membrane lipids composed of branched carbon chains attached to glycerol by ester linkage, use methionine as the start signal for protein synthesis, lack the rRNA loop that binds ribosomal protein in the eubacteria, and lack the common arm sequence (guanine-thymine-pseudouridine-cystine-guanine) of the tRNA found in all eukaryotes and eubacteria.

Least squares analysis of ribosomal RNA indicates two major groups of Archaea (Embley et al., 1994). One branch, the Crenarchaeota, includes a homogeneous group of sulfur-dependent extreme thermophiles. The other group, the Euryarchaeota, is more heterogeneous and includes methanogens (anaerobes with the ability to get energy by anaerobically biodegrading substances to methane—an

economically important biotechnology used worldwide both to reduce waste and generate fuel-grade biogas [Reeve, 1992]), extreme halophiles (Archaea living in high salt environments), and miscellaneous thermophiles (Woese, 1987). This classification is controversial and has inspired a limited debate over the best way to analyze DNA sequence data, as well as the appropriateness of relying on a single molecular sequence (in this case the ribosomal RNA) (see, for example, Lake, 1987, 1989; Rivera & Lake, 1992). Hopefully, as more data from different molecules are accumulated and systematists of prokaryotes become more sophisticated about phylogenetic analysis, a better substantiated hypothesis of archebacterial relationships will emerge.

The true Bacteria, or Eubacteria, have a peptidoglycan cell wall, membrane lipids composed of straight carbon chains attached to glycerol by ester linkages, an rRNA loop and the common arm sequence of the tRNA, and they use formylmethionine as a start signal for protein synthesis. Neighbor joining analysis indicates that there are approximately 13 groups of eubacteria (Embley et al., 1994), but because not all have been surveyed, those needing further investigation are temporarily placed in descriptive categories rather than formal taxa:

1. *Aquifex-Hydrogenbacter*—organisms that oxidize H_2 or reduce sulfur compounds at extreme temperatures (up to 95°C). Some authors have suggested that these may be phylogenetically intermediate between the remaining bacteria and the Archaea.
2. *Thermotogales*—two genera from geothermally heated marine sediments.
3. *Green nonsulfur or filamentous bacteria*—although most (e.g., *Chloroflexus*) are photosynthetic, a few of these thermophilic bacteria are nonphototrophic.
4. *Planctomycetales*—this group contains budding organisms that are often found attached to surfaces by holdfasts. These organisms have some unusual features for bacteria; at least one species has a membrane-bound nucleoid (Fuerst & Webb, 1991), and their rRNA operon is different from other bacteria in that the 16S and 23S genes are separate (Liesack & Stackebrandt, 1989; Liesack et al., 1992).
5. *Deinoccaceae/Thermus*—this group includes just two genera: the radiation resistant *Deinococcus* and the chemoorganotrophic thermophile *Thermus*, both of which have atypical cell walls containing ornithine in place of diaminopimelic acid in its peptidoglycan.
6. *Spirochaetes*—this group, which was originally recognized on the basis of its unique morphology and mode of motility, has been confirmed by analysis of rRNA.
7. *Chlamydia*—this group consists thus far of only one genus, *Chlamydia*, whose members are all intracellular parasites responsible for a number of sexually transmitted diseases and trachoma (a form of blindness).
8. *Actinomycetales*—gram-positive bacteria which may form branching chains that superficially resemble the filamentous bodies of fungi. They are notorious for species that cause tuberculosis and leprosy, but most are free-living soil microbes of interest to pharmaceutical companies for their ability to produce antibiotics that retard the growth of other bacteria.
9. *Proteobacteria*—this large, diverse group includes the purple sulfur bacteria and their relatives. Many of the organisms in this group are phototrophic and use the pigment bacteriochlorophyll, which absorbs longer wavelengths than other chlorophylls. It has been hypothesized (but not yet rigorously tested using phylogenetic methods) that the ancestor of this group was photosynthetic and that physiological diversity characteristic of the group arose as a result of the exchange of photosynthetic capacity for other physiological processes (such as sulfate reduction) as the bacteria expanded into new ecological niches. The group thus contains a diversity of bacteria including, in addition to the purple bacteria, sulfate- and sulfur-reducing bacteria, fruiting myxobacteria, enteric bacteria, free-living and symbiotic N_2 -fixers, and sulfide-oxidizing taxa.
10. *Bacteroides-Flavobacterium*—this group consists of several genera forming a major clade of gram-negative bacteria. It includes a mixture of physiological types including obligate anaerobes (e.g., *Bacteroides*) and obligate aerobes (*Sporocystophaga*).
11. *Gram-positive bacteria with low G+C%*—included here are the endospore formers, lactic acid bacteria, most cocci, the coryniform bacteria, and mycoplasmas.
12. *Cyanobacteria*—the blue-green algae, like eukaryotic plants, use chlorophyll *a* and two photosystems in tandem to transfer electrons from water to $NADP^+$ releasing O_2 as a waste product.
13. *Green sulfur bacteria*—these bacteria are photosynthetic but use only one photosystem to

transfer electrons from H_2S (rather than water) to $NADP^+$.

That these groups are natural phylogenetic lineages of Archaea and Bacteria still needs to be tested with cladistic analysis. Furthermore, the ability of the rRNA molecule to give significant support for the deep branches of these trees needs to be investigated.

EUKARYOTE DIVERSITY

Unicellular eukaryotic microorganisms are usually collectively referred to as protists. For many years, protistologists complained that an understanding of the phylogenetic relationships of the unicellular eukaryotes was hampered by the paucity of their fossil record and their microscopic size (see Corliss, 1974). These barriers are coming down, resulting in great research activity from which a new picture of protist relationships is emerging. The reasons for recent advances are twofold and obvious: First, with the widespread use of transmission electron microscopy, confocal microscopy, and molecular genetic sequencing, microscopic size is no longer a hindrance to gathering character data on these organisms. In fact, unicellular eukaryotic microorganisms possess a level of cellular and molecular diversity that far exceeds that found in multicellular eukaryotes, and this diversity provides a wealth of information for reconstructing their phylogeny. Second, systematists have refined their empirical methods—largely thanks to the cladistic revolution—and it is possible to sort through and analyze the new characters in meaningful ways.

One of the major changes that has emerged from these studies is the realization that the group is *not* a cohesive, taxonomic group that stands as an ancestral hub from which the other eukaryotic kingdoms are derived. Instead, the protista is paraphyletic, not monophyletic, and contains a heterogeneous mix of organisms at intermediate levels of organization and equivocal boundaries with multicellular taxa. A paraphyletic taxon is united by the possession of shared primitive characteristics (symplesiomorphies) rather than uniquely derived characteristics, and its members thus do not have ancestors unique to just themselves and lack unique individual histories (Hennig, 1966).

When unicellular forms are determined to be an organizational grade along lineages leading to multicellular taxa, the unicellular forms should be included in the group with their multicellular relatives. For example, there is no kingdom-level boundary between the unicellular chlorophytes and

the green plants, the choanoflagellates and the sponges, or the chrysophytes and the multicellular brown algae (Lipscomb, 1989). Not all unicellular forms are related to one of the multicellular kingdoms. There is evidence that some unicellular taxa (e.g., the kinetoplastids, euglenoids, or ciliates) are independent lineages and not related to the multicellular organisms, and these forms should be placed in their own separate taxonomic categories. Thus, it has become clear that commonly used 5-kingdom classification schemes and traditional categories (such as zooflagellate, phytoflagellate, and sarcodine) are oversimplified and simply inadequate for describing the true nature of diversity, and that multiple kingdoms are needed.

Determining what and how many of these categories there might be is not easy (see history of the field in Lipscomb, 1991). Cladistic analyses of the ultrastructural and biochemical features of the cell (Lipscomb, 1985, 1989, 1991, in prep.) confirmed the presence of at least thirteen major groups of protists, all of which, incidentally, had been proposed individually by earlier, more traditional systematists (e.g., Smith, 1951; Jeffrey, 1971; Leedale, 1974; Edwards, 1976; Taylor, 1976, 1978).

Some of these groups have traditionally been called algae because they are photosynthetic. Algae are not all phylogenetically related. In fact, the occurrence of at least six distinct lineages of algae provides immediate evidence for their biodiversity (Andersen, 1992):

1. *Rhodophyta*. The red algae, primarily because they can be multicellular and reach large body sizes, are persistently considered to be plants by many biologists even though their biology is quite different from that of green plants. They are characterized by chloroplasts bounded by two membranes containing separate thylakoids and lacking an external coat of endoplasmic reticulum (Dodge, 1973; Poeschel, 1989). They have chlorophyll *a*, α - and β -carotene, leutein, and zeaxanthin. Other pigments include the water-soluble allophycocyanin, phycocyanin, and phyrierythrin localized in phycobillosomes found on the thylakoids of the chloroplast. Floridean starch is the major storage product and it is found in the cytoplasm rather than the chloroplast. This carbohydrate has a primary chain of α -(1,4)-linked glucans with a 1,6-linked branched chain (Raven et al., 1990). There are no flagella, centrioles, or basal bodies (Poeschel, 1989). Without centrioles, it is not surprising to find that mitosis in red algae is different from that in green algae and

higher plants. The spindle forms on a unique nucleus associated organelle (called the NAO), which is located on each division pole. Furthermore, the nuclear membrane does not break down but remains intact except for small openings at the pole for the spindle to pass through (Scott, 1983). Red algae have the oldest fossil record of all the algae, with the bangiophytes found in 1.25-billion-year-old rocks (Butterfield et al., 1990). One cannot make too much of this because the fossil record for most early eukaryotes is very poor and some other protist group may have predated the red algae but left no record.

2. *Chlorobionts*. This clade represents one of the major groups of photosynthetic organisms. It includes the chlorophytes, charophytes, prasinophytes, and the multicellular plants. It has also been referred to as the chlorophyte series (Taylor, 1978) or the Viridiplantae (Cavalier-Smith, 1983). All of these taxa have chlorophylls *a* and *b*, and α -(1-4)-linked glucan (amylose/amylopectin) as a food storage in their chloroplasts. The chloroplast is bounded by two membranes, as in the rhodophytes, but the thylakoids are in many-layered grana. Motile cells with flagella have a stellate flagellar transition region and a cruciate flagellar root system. Most unicellular chlorobionts have a cell wall or scales, but not always made of cellulose.
3. *Cryptophytes* (= Cryptomonads) can be characterized as having chlorophyll *a* and *c*₂, phycobillins, and the xanthophyll alloxantin in chloroplasts surrounded by four membranes and thylakoids in stacks of two. The inner pair of membranes forms the plastid envelope and the outer pair forms the plastid endoplasmic reticulum. There is an expanded space between the plastid endoplasmic reticulum and the plastid envelope on one face. This expanded area contains ribosomes, starch grains, and the nucleomorph (a unique double membrane bound structure containing DNA). The nucleomorph has been postulated to be a vestigial nucleus belonging to a photosynthetic eukaryotic symbiont (Ludwig & Gibbs, 1987; Douglas et al., 1991). The food storage is the α -(1-4)-linked glucan glycogen, and it is stored in the nucleomorph. The mitochondrial cristae are flattened tubes. The flagella have tubular mastigonemes in two rows on one flagellum and one row on the other. They also possess a unique extrusome, called the refractile ejecto-

some, and a periplast of proteinaceous plates underlying the cell membrane.

4. *Chromobionts* (= Stramenopiles) are a group including both photosynthetic and heterotrophic forms. These are the heterokont unicellular organisms with tubular cristae in their mitochondria, mastigonemes in at least two rows on one of the flagella, and a β (1-3)-linked glucan as a food storage product. Those forms that are photosynthetic have chlorophyll *a*, *c*₁ and *c*₂, thylakoids in stacks of three, and four membranes surrounding the chloroplast with the outermost membrane continuing around the nucleus.

The placement of the oomycete and hyphochytrid fungi and the labyrinthulids with the chromobionts is not surprising and was suggested by mycologists (e.g., Barr, 1981, 1992). They share with the Raphidophyta a similar flagellar root structure that consists of a sheet of microtubules extending over the surface of the nucleus. All these taxa share with the gamete of foraminiferans the presence of mastigonemes in two rows on the forward projecting flagellum and a naked trailing flagellum.

The heliozoan Actinopodea appear to be derived from the chromobionts, specifically from the chrysophyte order Pedinellales. The Heliozoa were traditionally considered to be sarcodines because they have pseudopodia. These pseudopods, usually called axopodia, are long and slender and are made rigid by a core of microtubules. Ultrastructurally identical structures form an anterior ring around the flagella of the Pedinellales. Thus, presence of axopodia is a synapomorphy that unites the Heliozoa with the chrysophytes. This relationship has been reported before by protozoologists (e.g., Patterson, 1989) but often ignored by phycologists.

Loosely allied with the chromobionts are the Eustigmatophyta and the bicoecids. The Eustigmatophyta are photosynthetic and, as with other chromobionts, they have four membranes surrounding their chloroplast with thylakoids in stacks of three and two rows of mastigonemes on one flagellum. However, there is only chlorophyll *a* (no *c*) and the outermost chloroplast membrane is not continuous with the nuclear membrane. Whether the eustigmatophytes are primitive chromobionts or derived forms with many secondary losses cannot be determined at this time. The colorless, phagotrophic bicoecids have been considered to be related to the chromobionts by other protistologists be-

cause they also have two rows of mastigonemes on one flagellum, and their flagellar root structure is similar to that of the gametes of brown algae and the xanthophyte *Vaucheria* (Moestrup & Thomsen, 1976). However, they have evolved many unique features that are perhaps obscuring information that would allow us to place them definitively as the sister taxon to one of these chromophyte groups.

5. *Euglenozoa*. The euglenids and the kinetoplastid flagellates were first recognized as related taxa by Leedale (1967). The morphological features these taxa share include linked microtubules underlying the cell membrane, and discoidal cristae in the mitochondria. Some members of the Euglenida (or Euglenophyta in the botanical literature) are autotrophic and possess chlorophyll *a* and *b*. It has been hypothesized that these chloroplasts are the remnants of endosymbiotic chlorobionts or chlorobiont chloroplasts (Lefort-Tran, 1981; Whitley, 1981). Like many chlorophyte chloroplasts, those of the euglenids lack light-harvesting carotenoids (Rowan, 1989) and a girdle lamella. In addition, each chloroplast is surrounded by an additional single membrane (Dodge, 1973), which is consistent with the idea that they are remnant symbionts. Euglenids have a unique storage product, paramylon, which is a β (1,3)-linked glucan chain stored as grains in the cytoplasm. Phagotrophy is common in both photosynthetic and colorless forms, and the microtubules associated with the base of the flagella play a role in feeding (Triemer & Farmer, 1991).

6. *Alveolates*. The Dinoflagellata and Ciliophora have been linked as sister taxa on the basis of an alveolar membrane system and the presence of microtubules lining the cytopharynx. Because they also have alveolar membranes, the parasitic Apicomplexa are also included in this lineage. This alveolar membrane system consists of a layer of membrane-bound sacs lying just beneath the plasma membrane. In the dinoflagellates, these alveoli contain the theca. Some dinoflagellates are photosynthetic and so are sometimes called algae. These photosynthetic forms contain chlorophyll *a* and *c*₂ complexed with a unique xanthophyll, peridinin.

The Ciliophora make up one of the largest groups of protists and, as has already been discussed, play a major role in microbial food webs as predators and bacterivorous organisms. Ciliates generally have rows of cilia with a unique system of two microtubular and one fi-

brous roots. They also possess two kinds of nuclei: a micronucleus that functions in genetic exchange, and a macronucleus that functions in protein synthesis.

The Apicomplexa are all symbiotic and include many major disease-causing organisms (e.g., *Plasmodium*, which causes malaria). The anterior end of the cell contains a unique complex of organelles that presumably function in attachment and penetration of the hosts' cells.

The remaining groups consist almost exclusively of heterotrophic organisms. Many of these have been called protozoa, but this shared mode of nutrition is not sufficient to unite all of these forms into a taxonomic category.

7. *Parabasalida*. These heterotrophic flagellates all possess a distinctive flagellar root structure, which includes a rod of microtubules that extends from the basal bodies around the surface of the nucleus (= axostyle). An elaborate stack of golgi vesicles is often associated with this root between the nucleus and the basal bodies. These protists are almost exclusively symbiotic and are found in many different hosts. Some species, such as *Trichomonas*, are parasites of humans, but other species, such as *Barbulanympha* and *Trichonympha*, inhabit termites and the woodroach *Cryptocercus* where they aid in the digestion of cellulose.

8. *Metamonadida*. The majority of these flagellated protists are intestinal symbionts (e.g., *Giardia*), but some are free-living. They were once thought to be closely related to the parabasalans with whom they share the absence of mitochondria and storage glycogen, but they lack the axostyle and golgi apparatus. The metamonads are characterized by the presence of three bands of microtubules associated with the base of their flagella: a supranuclear band, an infranuclear band, and a band paralleling the recurrent flagellum and oral in-pocketing.

9. *Animals*. The taxa that are included in the animal clade are all united by mitosis in which the nuclear membrane breaks down by fragmentation, septate junctions, choanocytes, collagen, spermatozoa, and basal bodies at right angles to each other in flagellated cells. Grouping with the animal taxa are a protozoan taxon (the choanoflagellates) and the Chytridiomycota (chytrid fungi). The chytrids are grouped with the sponges and choanoflagellates because all have microtubules radiating perpendicularly to the basal body, which are interconnected by concentric rings of electron-

dense material (Lipscomb, 1989, 1991; Barr, 1992).

Although additional data is needed to be certain, it is probable that the Myxozoa, a group of protists with multicellular spores and specialized structures called polar capsules, will belong to the animal lineage. Like the true metazoans, they have a separation of somatic and germ cells and cell junctions. The polar capsules share many striking characteristics with the nematocysts of cnidarians (Lom, 1990), and these similarities appear to be synapomorphies that unite the Myxozoa with the cnidarian clade.

10. *Amoeboflagellates*. The amoeboflagellates and the cellular slime molds form a clade in which the taxa form limax amoeba that move relatively rapidly by the production of eruptive pseudopodia that lack fine extensions. This relationship has been suggested by other protozoologists (Page, 1976). The amoeboflagellates are also able to transform to a flagellated stage.
11. *Rhizopods*. The rhizopods with blunt lobose pseudopodia can be only tentatively grouped together. They have a simple cell structure and lack many of the characteristics used in the cladistic analysis. As the analysis is expanded, we may discover that this group is polyphyletic.
12. *Opalinids and Proteromonads*. These possess similar arrays of subpellicular microtubules underlying the folds in their cell membranes and a unique form of pinocytotic heterotrophy. Mignot (cited in the paper by Brugerolle & Joyon, 1975) was the first protozoologist to suggest that these forms were related.
13. *Microsporidia*. The members of this group have a number of features that are not apparently homologous to features found in other protists. They are small (2–7 μm) intracellular parasites that infect many kinds of eukaryotes, including other protists. The spore is the developmental stage with the longest duration, and at the light microscopic level it is the only life cycle stage that can be identified as belonging to a microsporidium. Not surprisingly then, most of our morphological information comes from the spore stage, which has a characteristic polar tube and cap used to inject sporoplasm into a host cell where it can grow and divide. Phylogenetic analysis of the small subunit rRNA often shows the microsporidians diverging first off of the eukaryote tree. This is most conservatively interpreted as meaning that the microsporidians are unique eukaryotes well

adapted to intracellular parasitism. But some have interpreted this to mean that the microsporidians are direct descendants of primitive eukaryotes (e.g., Cavalier-Smith, 1993).

In conclusion, a new classification consisting of at least 13 groups of eukaryotes is emerging, but a robust phylogeny of the eukaryotes will not be resolved until more taxa are examined with both morphological and molecular techniques. First, cell biology, particularly electron microscopy, has provided the basic data on which this multi-kingdom system is based, and many of these characters turn out to be convergent, or reversed, or both. For this reason, some branches are defined by very few characters and cladograms have several unresolved branch points where several lineages appear to diverge simultaneously rather than form a more informative branching pattern. Second, analyses do not yet include many parasitic and structurally unique forms. Thus they do not address phylogenetic placement of all of the taxa.

CONCLUSION

An appreciation and understanding of the natural world depends on a description of the systematic and ecological diversity of microorganisms as well as the better-known plants, animals, and fungi. There can be little doubt that the biodiversity of microorganisms is still poorly known. Yet as our knowledge has grown it has become increasingly clear that these organisms are an essential and diverse part of the ecosystems of the world.

There is no consensus on the phylogenetic relationships of the various unicellular organisms at the phylum and kingdom level and, as long as new taxa and characters are discovered, innovations in classifications of the kingdoms will likely continue. It is obvious that classifications that divide organisms into just two, three, four, or five kingdoms are too simplistic, and a multi-kingdom system provides a more realistic view of the diversity of life. Concomitant with this is the realization that some traditional categories such as monera, algae, protozoa, or fungi can no longer be considered distinct phylogenetic groups. An overall scheme of classification that reflects our growing databases has not yet completely emerged. Undoubtedly, it will not resemble those followed in many textbooks today, but it will reflect more accurately the relationships of the unicellular microorganisms.

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